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Metabolism of pyrimidine nucleotides in the deep-sea tube worm *Riftia pachyptila* and its bacterial endosymbiont

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Introduction

Riftia pachyptila Jones, 1981, is a tubeworm found only in the close vicinity of deep-sea hydrothermal vents in the Pacific ocean (Gaill, 1993). The organism thrives in a community that is almost completely isolated from the biosystems of the rest of the planet. In the vent environment these animals encounter both physical and chemical obstacles, such as elevated pressure, high temperature and chemical toxicity.

In this organism the only tissue in direct contact with the surrounding water is the plume which has a large highly vascularized surface area, allowing an efficient exchange of metabolites and waste products between the environment and the animal. The remaining tissues are within the *Riftia* tube. The vestimentum is a muscle the animal uses to position itself in the tube. Within the large sac made by the body wall and terminated by the opisthosome are two major tissues of the worm: one, the coelomic fluid, bathes the other, the trophosome (Gaill, 1993).

The trophosome tissue is densely colonized by a sulfur-oxidizing chemoautotrophic endosymbiotic bacterium (Cavanaugh et al., 1981; Felbeck, 1981; Nelson & Fisher, 1995). The bacterial volume is estimated to be between 15 and 35% of the total volume of the trophosome (Cavanaugh et al., 1981; Powell & Somero, 1986). All metabolite exchanges between the trophosome and the sea water surrounding the worm are mediated via the blood in the vascular system which contains a circulating haemoglobin (Zal, 1996). The most obvious functions of the blood are to supply host tissues with oxygen and to transport CO_2, O_2, H_2S and minerals to the bacteria. In return, the bacteria produce metabolic energy from the oxidation of hydrogen sulphide and provide organic compounds to the worm. Thus, this particular nutritional organization involves specific metabolic exchanges between the two organisms.

All living organisms rely on two metabolic pathways for the production of pyrimidine nucleotides. The *de novo* pathway allows the complete synthesis of these nucleotides including the synthesis of the pyrimidine ring starting with bicarbonate, glutamine and ATP. The salvage pathway ensures the production of these nucleotides from the pyrimidine nucleosides and nucleotide monophosphates provided by the intracellular degradation of nucleic acids. Alternatively, these degradation products (nucleotide monophosphates and nucleosides) can be degraded by enzymes of catabolic pathways (Borel et al., 1997). Consequently, these products of degradation of pyrimidine nucleotides can represent a possible source of carbon and nitrogen for the organism.

Here we summarize the information that we could obtained concerning the organization of the *de novo* and salvage pyrimidine pathways as well as the catabolic pathways in *Riftia pachyptila* and its bacterial endosymbiote.

Materials and methods

Source and storage of Riftia pachyptila *samples*. Samples of *R. pachyptila* were collected in the eastern part of the Pacific Ocean at a depth of 2600 m (Hope 99), using the submersible "Nautile", and recovered in an isothermal box for the trip to the surface. To avoid interference with the subsequent enzymatic tests, the specimens were immediately bled and dissected on board, and isolated organs frozen in liquid nitrogen as previously described (Simon et al., 2000; Minic et al., 2001).

Purification of the bacterial symbiont. Immediately after collecting and bleeding the animal, the bacterial symbiont was purified by the method proposed by Distel & Felbeck (1988), under the conditions previously described (Simon et al., 2000; Minic et al., 2001).

Protein extract from each organ of Riftia pachyptila. Protein extracts from all organs were freshly prepared before the enzyme assays. Frozen tissue (ca. 2 g) was suspended in 6 ml of ice-cold extraction buffer (Minic et al., 2001, 2002). The mixture was homogenized in a Potter homogenizer (Teflon pestle). The homogenate was further disrupted by sonication three times for 60 s each with a Biosonik III sonicator at 20 kcycles s^{-1}. The homogenate was then centrifuged at 9000 g for 20 min and the resulting supernatant was utilized for subsequent enzymatic assays.

Enzymatic assays. The enzyme activities were measured by previously described methods (Minic et al., 2001, 2002).

Total protein assay. Total protein concentration was determined by the Lowry method (Lowry et al., 1951), with bovine serum albumin dissolved in extraction buffer as the standard.

Abbreviations

ATCase, aspartate transcabamylase; CPSase, carbamylphosphate synthetase; CDase, cytidine deaminase; CKase, cytidine kinase; CTPSase, CTP-synthetase; DHOase, dihydroorotase; DHODase, dihydroorotate dehydrogenase; Gln, glutamine; OPRTase, orotatephosphoribosyl transferase; uracilPRTase, uracilphosphoribosyl transferase; uracilRase, uracil reductase; UKase, uridine kinase. UPase, uridine phosphorylase.

Results

Distribution of enzyme activities of the de novo *pyrimidine nucleotides pathway in the different parts of* Riftia pachyptila

Since it was previously shown that CPSase and ATCase, the first two enzymes of the *de novo* pyrimidine pathway, are present only in the trophosome (Simon et al., 2000), the distribution of the subsequent enzymes of this pathway in the different parts of the worm was examined. The results obtained are presented in Table 1. Interestingly, it appears that the third enzyme of this pathway, DHOase, is also present only in the trophosome, the symbiont-harbouring tissue. In contrast, the next two enzymes (DHODase, OPRTase) and the last enzyme of the pathway, CTP synthase (CTPSase), are present in all organs of the animal. The fact that the first three enzymes are present only in the trophosome raised the question of whether these enzymes belong to the bacteria or to the worm. This point was further investigated.

Table 1. Activities of the enzymes of the *de novo* pyrimidine pathway in the different tissues and in the isolated bacterial symbiont of *Riftia pachyptila*. All specific enzyme activities were measured at 37°C (Minic et al., 2001). The numbers between brackets indicate the number of determinations made on different *Riftia* individuals. Abbreviations: nd, not detected; *, determined by Simon et al., 2000.

Body part	Enzyme activities (nmol min^{-1} mg^{-1} protein)					
	CPSase(Gln)	ATCase	DHOase	DHODase	OPRTase	CTPSase
Branchial plume	nd	nd	nd	32 ± 5 (3)	0.072 ± 0.004 (3)	0.225 ± 0.026 (3)
Vestimentum	nd	nd	nd	26 ± 6 (3)	0.037 ± 0.003 (3)	0.204 ± 0.012 (3)
Trophosome	1.85 ± 0.10 (3)*	4.2 ± 1.0 (10)*	0.58 ± 0.19 (3)	24 ± 4 (3)	0.147 ± 0.013 (3)	0.016 ± 0.010 (3)
Body wall	nd	nd	nd	53 ± 7 (3)	0.112 ± 0.009 (3)	0.124 ± 0.048 (3)
Opisthosome	nd	nd	nd	20 ± 6 (3)	0.180 ± 0.015 (3)	0.196 ± 0.090 (3)
Isolated bacteria	nd	0.076 ± 0.064 (3)	2.4 ± 1.4 (4)	164 ± 40 (3)	0.072 ± 0.007 (2)	0.142 ± 0.017 (2)

Enzymatic activities in the isolated symbiotic bacteria

The same enzymatic determinations were made on extracts from bacteria isolated aboard, immediately after collection of the animals. The results of this analysis are given in Table 1. In the bacterial extract all the enzyme activities analysed were detected except for CPSase(Gln). The instability of CPSases is well known, and most probably this enzyme was inactivated during the isolation and/or the storage of the bacterial preparations. The presence of ATCase and DHOase and their catalytic and regulatory properties (including for CPSase) confirm the hypothesis of the bacterial origin of these enzymes in the trophosome (Minic et al., 2001). The OPRTase, DHODase and CTPSase activities are also present in the isolated bacteria. Thus, it appears that, in contrast to the worm, the bacteria possess all the enzymes of the *de novo* pyrimidine pathway.

Distribution of enzyme activities of the salvage pathways in the different parts of Riftia pachyptila

The results presented above indicate that the worm is unable to synthesize the pyrimidine nucleotides through the *de novo* pathway. Thus, it must rely on the salvage pathway. Consequently, we investigated whether the enzymes of this pathway are present in the different parts of the worm. The results of these analyses are given in Table 2. CDase, UKase, and uracilPRTase are present in all tissues of the host. Unexpectedly, the isolated bacteria did not exhibit activity for any enzyme of the salvage pathway studied. Complementary biochemical and kinetic analyses were performed in order to obtain information about the origin of the enzymes of the salvage pathways in the trophosome. The results obtained indicate that the enzymes of the salvage pathways present in the trophosome belong to the host (Minic et al., 2001).

Distribution of enzyme activities of pyrimidine catabolism in the different tissues of Riftia pachyptila

The results of analysis of the distribution of 5'-nucleotidase, UPase and UracilRase activities are given in Table 3. All three enzymes were present in all tissues of the host.

Table 2. Activities of the enzyme of the "salvage" pyrimidine pathways in the different tissues of *Riftia pachyptila***.** All enzyme activities were measured at 37°C (Minic et al., 2001). The numbers between brackets indicate the number of determinations made on different *Riftia* individuals. Abbreviation: nd, not detected.

	Enzyme activities (nmol min^{-1} mg^{-1} protein)		
Body part	CDase	UKase	UracilPRTase
Branchial plume	0.020 ± 0.003 (3)	0.053 ± 0.032 (3)	0.035 ± 0.013 (3)
Vestimentum	0.054 ± 0.004 (3)	0.192 ± 0.073 (3)	0.026 ± 0.014 (3)
Trophosome	0.038 ± 0.003 (3)	0.107 ± 0.030 (3)	0.014 ± 0.003 (3)
Body wall	0.292 ± 0.024 (3)	0.958 ± 0.617 (3)	0.035 ± 0.007 (3)
Opisthosome	0.121 ± 0.024 (3)	0.084 ± 0.065 (3)	0.033 ± 0.007 (3)
Isolated bacteria	nd	nd	nd

Table 3. Distribution of specific enzymes that participate in the catabolism of pyrimidine nucleotides in the host tissues and isolated bacteria of *Riftia pachyptila***.** All specific enzyme activities were measured at 37°C (Minic et al., 2002). The mean and standard deviation were calculated for three different *R. pachyptila* extracts. Abbreviation: nd, not detected.

Body part	Specific activities (nmol min^{-1} mg^{-1} protein)			
	5'-Nucleotidase		UPase	uracilRase
	UMP	CMP		
Branchial plume	0.045 ± 0.044	0.085 ± 0.074	0.783 ± 0.158	1.63 ± 0.57
Vestimentum	0.081 ± 0.033	0.107 ± 0.016	1.578 ± 0.155	1.76 ± 0.73
Trophosome	0.065 ± 0.027	0.075 ± 0.029	0.335 ± 0.108	0.78 ± 0.38
Body wall	0.007 ± 0.005	0.012 ± 0.005	7.327 ± 1.724	1.38 ± 0.39
Opisthosome	0.313 ± 0.283	0.456 ± 0.331	0.201 ± 0.085	1.64 ± 0.59
Isolated bacteria	nd	nd	nd	nd

Unexpectedly, the isolated bacteria did not exhibit any activity for the enzymes of the catabolic pathways, a result which was also confirmed by complementary biochemical and kinetic experiments (Minic et al., 2002).

Discussion

The results reported above emphasize the specific metabolic organization and interdependence between the host and the bacterial symbiont for the biosynthesis and catabolism of pyrimidines in *R. pachyptila*. Figure 1 shows the model which assembles the results of this investigation and describes the general organization of the pyrimidine nucleotides metabolism in *R. pachyptila*.

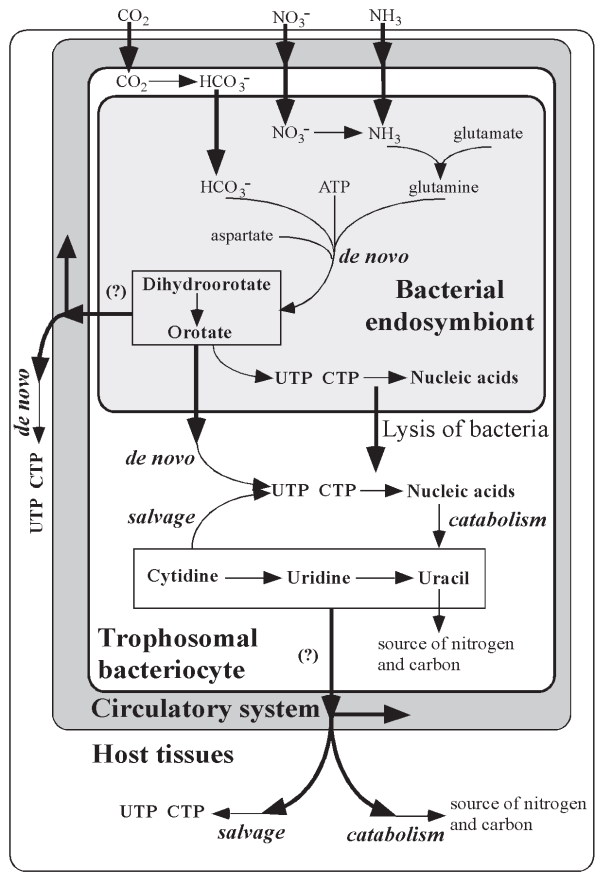

Figure 1. Integrated scheme of pyrimidine nucleotides metabolic pathways in *Riftia pachyptila*. The model is based on the distribution and properties of enzymes of the pyrimidine anabolism and catabolism, and the exchanges between the endosymbiont and the trophosomal host cells and other host tissues of *R. pachyptila*. *Question marks* indicate steps that have not been completely elucidated. *Thin arrows* refer to metabolic pathways. *Thick arrows* refer to transport of metabolites in compartments, tissues of body parts.

The symbiotic bacterium possesses enzymatic equipment for the biosynthesis of pyrimidine nucleotides through the *de novo* pathway, but lacks the enzymes of the salvage and catabolic pathways (Minic et al., 2001, 2002). In contrast, the host cells (including the bacteriocytes) possess the enzymes catalyzing the final steps of the *de novo* pathway as well as the enzymatic equipment for the salvage pathways leading to the synthesis of pyrimidines from nucleic acid degradation products. Since the host cells do not have the first three enzymes of the *de novo* pathway (CPSase, ATCase and DHOase), the necessary metabolic precursors, orotate and/or dihydroorotate, must be provided by the bacteria. The first reaction of this *de novo* pathway (carbamyl-phosphate synthetase) relies on inorganic carbon and nitrogen provided by the external medium. Thus, the *de novo* pathway to pyrimidine nucleotides in *R. pachyptila* is absolutely dependent on the symbiotic bacteria. For this reaction to occur CO_2, NH_3, and nitrate are provided by the

external environment. Nitrate is reduced by assimilatory enzymes present only in the bacteria (Minic et al., 2001; Hentschel and Felbeck, 1992; Girguis et al., 2000). The resulting NH_3, is used for the synthesis of glutamine from glutamate, glutamine being the substrate of the carbamyl-phosphate synthetase specific to the pyrimidine pathway and present only in the bacteria (Simon et al., 2000; Minic et al., 2001).

The results presented here show that *R. pachyptila* possesses the activities of three enzymes participating in the catabolism of pyrimidine nucleotides, 5'-nucleotidase, UPase and uracilRase, in all its tissues. Notably, these enzymes do not exist in the bacterial endosymbiont. Catabolism of pyrimidine nucleotides leads to the production of CO_2, NH_3, malonyl-CoA and succinyl-CoA; subsequently malonyl-CoA can be used for the biosynthesis of fatty acids while succinyl-CoA enters into the citric acid cycle (Borel et al., 1997). In this manner the degradation of pyrimidine nucleotides can represent an alternative nutritional source of nitrogen and carbon, besides the external environment of the worm, and also can feed other biosynthetic pathways. This degradation is consistent with the reported bacterial lysis in the trophosome (Bright et al., 2000).

The study of the localization of these anabolic and catabolic enzymes in the trophosome shows that they are not homogenously distributed, and suggest some kind of structural and physiological organization of this tissue (Minic et al., 2002).

In conclusion, the two symbiotic partners in *R. pachyptila* have developed a particular metabolic organization and a nutritional strategy involving numerous interactions and metabolic exchanges as shown here in the particular case of pyrimidine metabolism. This complex organization is the basis of the adaptation of *R. pachyptila* to the extreme hydrothermal vent environment and the absence of readily available source of organic carbon through photosynthesis.

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